Journal of Chemical Ecology, Vol. 19, No. 10, 1993

HERBICIDAL ACTIVITY OF SULFORAPHENE FROM STOCK (*Matthiola incana*)[1]

ANITA M. BRINKER[2] and GAYLAND F. SPENCER*

Bioactive Constituents Research
National Center for Agricultural Utilization Research, Agricultural Research Service, USDA
1815 N. University St., Peoria, Illinois 61604

(Received March 4, 1993; accepted May 10, 1993)

Abstract—A herbicidal compound was isolated from extracts of *Matthiola incana* and identified as sulforaphene (4-methylsulfinyl-3-butenyl isothiocyanate). The ED_{50} of this compound against velvetleaf seedlings was approximately 2×10^{-4} M. Glucoraphenin, the glucosinolate that is the natural precursor of sulforaphene, was less phytotoxic, with an ED_{50} of near 6×10^{-3} M.

Key Words—Stock, *Matthiola incana*, Brassicaceae, glucosinolate, sulforaphene, glucoraphenin, phytotoxicity, allelopathy.

INTRODUCTION

Anecdotal reports (Rice, 1984) indicate that greenhouse soil in which stock (*Matthiola* and *Malcolmia* spp.) has been grown cannot be reused, suggesting that these species might be allelopathic. Both genera are members of the Brassicaceae, which includes other species reported to have phytotoxic activity (Bialy et al., 1990; Brown et al., 1991). Our interest in naturally occurring compounds with herbicidal activity prompted us to determine whether stock produces herbicidal substances.

*To whom correspondence should be addressed.

[1] The mention of firm name or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

[2] Present address: Westfälische Wilhelms-Universität, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstrasse 56, D-4400, Münster, Germany.

0098-0331/93/1000-2279$07.00/0

METHODS AND MATERIALS

Spectroscopy. ^1H and ^{13}C NMR spectra relative to tetramethylsilane were measured in $CDCl_3$ or D_2O at 300 and 75.5 MHz, respectively, with a Bruker WM-300-WB spectrometer. Mass spectra (70 eV) were produced by a Hewlett Packard 5790 Mass Selective Detector.

Preparation of Extracts. Seeds of cv. Happi-stok [*Matthiola incana* (L.) R.Br.] were purchased from Thompson and Morgan, Inc. (Jackson, New Jersey). Two-month-old, greenhouse-grown plants (not flowering) were cut off at soil level, rinsed in water, blotted dry, and weighed. The above ground plant parts (about 300 g fresh wt) were ground in a Waring blender with 300 ml chloroform, and the extract was filtered through cheesecloth. The grinding and filtration steps were repeated twice. The filtrates were combined, filtered through Whatman 2 paper, and washed three times with water in a separatory funnel. The chloroform layer was concentrated under vacuum to a thick liquid and then diluted with a few milliliters of chloroform; the aqueous layer was freeze-dried and redissolved in water. The remaining solid plant material was ground again three times with methanol (300 ml each time); these extracts were combined and filtered as above, and concentrated until almost dry.

Roots (about 20 g) were washed with distilled water to remove dirt, then extracted as above.

Chromatography. The chloroform extract of the leaves and stems (about 3.5 g) was chromatographed on a 6 × 62-cm column of Sephadex LH-20; the eluent was dichloromethane–methanol, 1 : 1 (v/v). Seven fractions corresponding to colored bands were collected. The band found to be active by bioassay (see below) was further chromatographed on 1-mm-thick silica gel 60 plates (Merck) in chloroform–acetone, 2 : 1 (v/v). Zones (identified by fluorescent bands and the areas between them) were eluted with acetone followed by chloroform. The active zone yielded sulforaphene. ^1H NMR ($CDCl_3$): δ 2.6–2.7 (2H, m, $C\underline{H}_2$); 2.65 (3H, s, $C\underline{H}_3$); 3.64 (2H, t, J = 6 Hz, $C\underline{H}_2$—NCS; 6.35–6.5 (2H, m, $C\underline{H}$=$C\underline{H}$). ^{13}C ($CDCl_3$): δ 32.61 ($\underline{C}H_2$); 40.50 ($\underline{C}H_3$); 43.59 ($\underline{C}H_2$—NCS); 133.20 and 137.93 ($\underline{C}H$=$\underline{C}H$); 177.5 (N$\underline{C}$S).

Isolation of Glucoraphenin (4-Methylsulphinyl-3-butenyl Glucosinolate). Radish (*Raphanus sativus* L.) seeds purchased commercially were ground in a Wiley mill and extracted overnight with hexane in a Soxhlet extractor to remove the lipids. The defatted meal (about 70 g) was added to 300 ml of boiling methanol and heated on a steam bath for 3 min. The mixture was filtered through Whatman 2 paper; the solid was boiled again for 3 min in 300 ml of 75% aqueous methanol, then filtered. The filtrates were combined, filtered through Whatman 1, and concentrated under vacuum. Fifty milliliters of lead acetate–barium acetate (0.5 M each) was added and then enough water to bring the total volume to 500 ml. The mixture was centrifuged (2300 rpm, 10 min) and the

pellet discarded. The supernatant was chromatographed on a column of Sephadex LH-20 with water; the effluent was monitored by a UV detector (226 nm). The eluate corresponding to a large peak due to glucoraphenin was collected and freeze-dried. The solid residue was dissolved in ethanol, filtered, and concentrated to dryness under a stream of nitrogen. ^{1}H NMR (D_2O): δ 2.65–2.8 (2H, m, $C\underline{H}_2$); 2.73 (3H, s, $C\underline{H}_3$); 2.94 (2H, t, J = 7 Hz, $C\underline{H}_2$); 3.4–4.0 (glucose), 5.05 (1H, d, J = 10 Hz, H-1 of glucose); 6.53–6.6 (2H, m, $C\underline{H}=C\underline{H}$). ^{13}C NMR ($D_2O$): δ 29.68, 31.53 ($\underline{C}H_2$); 39.80 ($\underline{C}H_3$); 61.71 (glucose C-6); 70.22 (glucose C-4); 73.00 (glucose C-2); 78.10 (glucose C-3); 81.16 (glucose C-5); 82.73 (glucose C-1); 133.99, 142.40 ($\underline{C}H=\underline{C}H$); 163.28 (quaternary C). These resonances were assigned by comparison with the data of Linscheid et al. (1980) and Cox et al. (1984).

Bioassay. Velvetleaf (*Abutilon theophrasti* Medic.) seeds (Valley Seed Service, Fresno, California) were soaked in 10% Clorox for 15 min, then in water for about 4 hr. They were incubated in darkness overnight in Petri dishes lined with wet filter paper. Test solutions were added to 2.5-ml agar solutions (6 g dry agar per liter) in 50 × 9-mm plastic Petri dishes. Each experiment included control dishes with solvent alone. All crude extracts were assayed at 1 mg extract/ml agar, except for the chloroform and aqueous extracts of the aboveground plant parts; they were assayed at 2 mg/ml. Column fractions were also assayed at 1 mg of each fraction per milliliter agar. After the agar had cooled and the solvent had evaporated from the agar, four to five seedlings were placed in each dish. The dishes were covered and incubated in darkness overnight, then evaluated visually for inhibition of seedling and root growth.

Solutions of glucoraphenin (dissolved in water) and sulforaphene (in acetone) were assayed as above, but with 2 ml agar per Petri dish (two dishes at each concentration). Radical lengths (10 per concentration) were measured after 48 hr of incubation. ED_{50} values were estimated from the graphed results (see below).

RESULTS AND DISCUSSION

Identification of Phytotoxic Component. The crude chloroform extract of aboveground plant parts caused visible inhibition of velvetleaf seedling growth; the aqueous wash following chloroform extraction and the chloroform extract of roots were slightly active. Other extracts were not inhibitory. Fractionation of the chloroform extract of aboveground parts on Sephadex LH-20 gave one fraction that completely inhibited the growth of velvetleaf seedlings. When this fraction was chromatographed on thin-layer plates and the chromatograms bioassayed, the activity corresponded to a zone (R_f = 0.4–0.5) in which no spot was evident under either visible or UV light. The remainder of this fraction was

chromatographed on a preparative TLC plate, and the zone of activity was eluted with $CHCl_3$. Comparison of IR, EI-MS, and ^1H NMR data for the eluate with literature data (Balenovic et al., 1966; Spencer and Daxenbichler, 1980; Kawabata et al., 1989) indicated that the active compound was sulforaphene (4-methylsulfinyl-3-butenyl isothiocyanate), a hydrolysis product of the glucosinolate glucoraphenin (Figure 1). This compound has been reported as the major glucosinolate in seed and green parts of *Matthiola fruticulosa* (L). Maire [= *M. tristis* (L.) R. Br.; Matas, 1960; Gmelin and Kjaer, 1970; Daxenbichler et al., 1991] and in seed of *M. parviflora* (Schousboe) R. Br., *M. bicornis* (Sibth and Sm.) DC., and *M. sinuata* (L.) R. Br. (Daxenbichler et al., 1991).

Toxicity of Sulforaphene and Glucoraphenin. The sulforaphene sample from stock and the glucoraphenin isolated from radish seeds were assayed against velvetleaf seedlings to determine the phytotoxicity of these compounds. The ED_{50} of sulforaphene was approximately 2×10^{-4} M (Figure 2). A similar value was obtained when the compound was assayed with wheat seedlings (data not shown). This value is comparable to, or lower than, values obtained for several phenolic compounds implicated in allelopathy of red clover (Chang et al., 1969) and yellow fieldcress (Yamane et al., 1992). Glucoraphenin was considerably less active against velvetleaf; its ED_{50} was about 6×10^{-3} M (Figure 2).

Sulforaphene has not previously been reported to be biologically active, although the structurally similar compound 4-methylthio-3-butenyl isothiocyanate is antimicrobial (Esaki and Onozaki, 1982), and 8-methylsulfinyloctyl, 9-methylsulfinylnonyl, and 10-methylsulfinyldecyl isothiocyanates are herbicidal (Kawabata et al., 1989; Yamane et al., 1992). Sulforaphene would appear to be more active than other herbicidal isothiocyanates (Bialy et al., 1990). Furthermore, 4-methylthio-3-butenyl isothiocyanate degrades quickly in water (Esaki and Onozaki, 1982); if this is also true for sulforaphene, our assays may have underestimated its toxicity. That glucoraphenin is less active than sulfor-

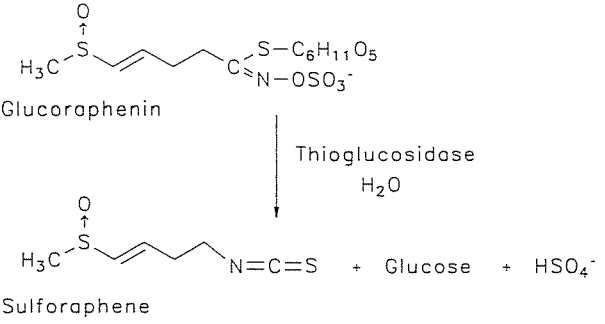

FIG. 1. Enzymatic hydrolysis of glucoraphenin to sulforaphene.

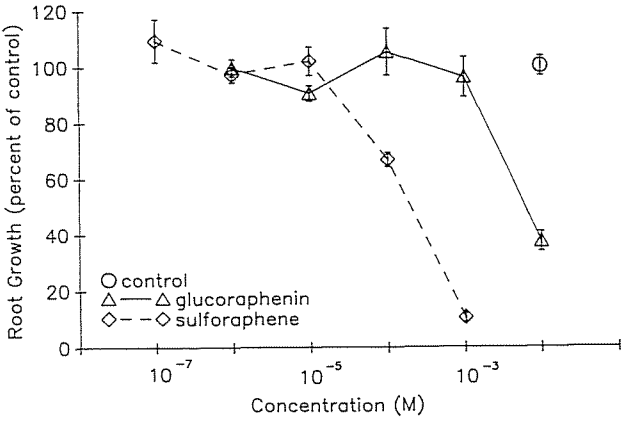

FIG. 2. Inhibition of velvetleaf seedling root growth by glucoraphenin and sulforaphene. Vertical bars represent ± 1 SE.

aphene is not surprising; isothiocyanates are generally more phytotoxic than intact glucosinolates (Bialy et al., 1990). It would appear that the isothiocyanate moiety is important for activity. Thus, any phytotoxicity due to *M. incana* or other *Matthiola* species is probably caused by sulforaphene resulting from the breakdown of glucoraphenin in soil, rather than by glucoraphenin acting directly. Measures were not taken during extraction to prevent hydrolysis of glucoraphenin, and it is assumed that sulforaphene is present in vivo at very low levels, if at all. These particular compounds are comparatively uncommon in nature and thus have not been much studied for biological activity even though glucoraphenin is the dominant glucosinolate in radish seed (Sang et al., 1984; Daxenbichler et al., 1991).

Acknowledgments—We thank Joanne Toohill for growing the plants, David Weisleder for the NMR spectra, and Ronald Plattner for the mass spectrum.

REFERENCES

BALENOVIC, K., DELJAC, A., MONKOVIC, I., and STEFANAC, Z. 1966. Synthesis of (±)-sulphoraphene. *Tetrahedron* 22:2139–2143.

BIALY, Z., OLESZEK, W., LEWIS, J., and FENWICK, G.R. 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. *Plant Soil* 129:277–281.

BROWN, P.D., MORRA, M.J., MCCAFFREY, J.P., AULD, D.L., and WILLIAMS, III, L. 1991. Allelochemicals produced during glucosinolate degradation in soil. *J. Chem. Ecol.* 17:2021–2034.

CHANG, C.-F., SUZUKI, A., KUMAI, S., and TAMURA, S. 1969. Chemical studies on "clover sick-

ness." Part II. Biological functions of isoflavonoids and their related compounds. *Agric. Biol. Chem.* 33:398–408.

COX, I.J., HANLEY, A.B., BELTON, P.S., and FENWICK, G.R. 1984. N.M.R. spectra (^{1}H, ^{13}C) of glucosinolates. *Carbohydr. Res.* 132:323–329.

DAXENBICHLER, M.E., SPENCER, G.F., CARLSON, D.G., ROSE, G.B., BRINKER, A.M., and POWELL, R.G. 1991. Glucosinolate composition of seeds from 297 species of wild plants. *Phytochemistry* 30:2623–2638.

ESAKI, H., and ONOZAKI, H. 1982. Antimicrobial action of pungent principles in radish root. Eiyo To Shokuryo 35:207–211.

GMELIN, R., and KJAER, A. 1970. Glucosinolates in *Matthiola fruticulosa* and related species: A reinvestigation. *Phytochemistry* 9:569–573.

KAWABATA, J., FUKUSHI, Y., HAYASHI, R., SUZUKI, K., MISHIMA, Y., YAMANE, A., and MIZUTANI, J. 1989. 8-Methylsulfinyloctyl isothiocyanate as an allelochemical candidate from *Rorippa sylvestris* Besser. *Agric. Biol. Chem.* 53:3361–3362.

LINSCHEID, M., WENDISCH, D., and STRACK, D. 1980. The structures of sinapic acid esters and their metabolism in cotyledons of *Raphanus sativus*. *Z. Naturforsch.* 35C:907–914.

MATAS, L.C. 1960. Sobre los compuestos senevolicos de *Matthiola tristis* (L.) R. Br. *Farmacognosia* 20:307–313.

RICE, E.L. 1984. Allelopathy, 2nd. ed. Academic Press, New York, p. 117.

SANG, J.P., MINCHINTON, I.R., JOHNSTONE, P.K., and TRUSCOTT, R.J.W. 1984. Glucosinolate profiles of the seed, root and leaf tissue of cabbage, mustard, rapeseed, radish and swede. *Can. J. Plant Sci.* 64:77–93.

SPENCER, G.F., and DAXENBICHLER, M.E. 1980. Gas chromatography–mass spectrometry of nitriles, isothiocyanates and oxazolidine-thiones derived from cruciferous glucosinolates. *J. Sci. Food Agric.* 31:359–367.

YAMANE, A., FUJIKURA, J., OGAWA, H., and MIZUTANI, J. 1992. Isothiocyanates as allelopathic compounds from Rorippa indica Hiem. (Cruciferae) roots. *J. Chem. Ecol.* 18:1941–1954.